

WHAT IS CLAIMED IS:

- 1 1. A method of regulating the replication of a DNA molecule, the
2 method comprising,
3 introducing into a eukaryotic cell,
4 a) a replication cassette comprising an origin of replication; and
5 b) a replication system comprising
6 i) a polynucleotide encoding a polypeptide with RNA
7 polymerase activity;
8 ii) a polynucleotide encoding a polypeptide with DNA
9 polymerase activity;
10 iii) a polynucleotide encoding a polypeptide with DNA
11 helicase activity and;
12 iv) a polynucleotide encoding a polypeptide with DNA
13 primase activity;
14 wherein the polynucleotide encoding each polypeptide is operably
15 linked to a eukaryotic replication promoter, thereby initiating replication of the replication
16 cassette independent from chromosomal DNA replication.
- 1 2. The method of claim 1, wherein the replication system comprises a
2 polynucleotide encoding each of the following polypeptides: T7 RNA polymerase, T7
3 gene 4 protein, T7 DNA polymerase and TrxA.
- 1 3. The method of claim 2, wherein the eukaryotic cell is a plant cell.
- 1 4. The method of claim 2, wherein the eukaryotic cell is a mammalian
2 cell.
- 1 5. The method of claim 2, wherein the origin of replication is a T7
2 bacteriophage origin of replication.
- 1 6. The method of claim 2, wherein the replication cassette comprises
2 a T7 promoter.
- 1 7. The method of claim 2, wherein the replication cassette comprises
2 an expression cassette.

1 8. The method of claim 2, wherein the expression cassette comprises
2 a polynucleotide operably linked to an expression promoter in an antisense orientation.

1 9. The method of claim 2, wherein the expression cassette comprises
2 a polynucleotide operably linked to an expression promoter in a sense orientation.

1 10. The method of claim 2, wherein the replication cassette comprises
2 at least 200 base pairs of DNA that is at least 70% identical to chromosomal DNA in the
3 eukaryotic cell.

1 11. The method of claim 10, wherein the replication cassette comprises
2 at least 200 base pairs of DNA that is identical to chromosomal DNA in the eukaryotic
3 cell.

1 12. The method of claim 2, wherein the replication cassette comprises
2 a recombination sequence.

1 13. The method of claim 12, wherein the recombination sequence is a
2 *lox* sequence.

1 14. The method of claim 2, wherein the replication system
2 polynucleotide(s) further encode a sequence-specific recombinase operably linked to a
3 promoter.

1 15. The method of claim 14, wherein the sequence-specific
2 recombinase is the Cre recombinase.

1 16. The method of claim 2, wherein the eukaryotic replication
2 promoter is tissue-specific.

1 17. The method of claim 2, wherein the eukaryotic replication
2 promoter is constitutive.

1 18. The method of claim 2, wherein the eukaryotic replication
2 promoter is meiosis-specific.

1 19. The method of claim 2, wherein the eukaryotic replication
2 promoter is inducible.

1 20. The method of claim 2, wherein at least one of the replication
2 system polynucleotide(s) encoding T7 RNA polymerase, T7 gene 4 protein, T7 DNA
3 polymerase and TrxA encode a nuclear localization signal.

1 21. The method of claim 20, wherein all of the replication system
2 polynucleotide(s) encoding T7 RNA polymerase, T7 gene 4 protein, T7 DNA polymerase
3 and TrxA encode a nuclear localization signal.

1 22. The method of claim 2, wherein the number of copies of the
2 replication cassette is increased.

1 23. A eukaryotic organism comprising a polynucleotide encoding each
2 of the following polypeptides: T7 RNA polymerase, T7 gene 4 protein, T7 DNA
3 polymerase and TrxA, wherein the polynucleotide encoding each polypeptide is operably
4 linked to a eukaryotic replication promoter.

1 24. The eukaryotic organism of claim 23, wherein the organism is a
2 plant.

1 25. The plant of claim 24, further comprising a replication cassette
2 comprising a bacteriophage T7 origin of replication.

1 26. The plant of claim 24, wherein the replication cassette comprises
2 an expression cassette.

1 27. The plant of claim 24, wherein the expression cassette comprises a
2 polynucleotide operably linked to an expression promoter in an antisense orientation.

1 28. The plant of claim 24, wherein the expression cassette comprises a
2 polynucleotide operably linked to an expression promoter in a sense orientation.

1 29. The plant of claim 24, wherein the replication cassette is episomal.

1 30. The plant of claim 29, wherein the replication cassette is a plasmid.

1 31. The plant of claim 24, wherein the replication cassette is integrated
2 into a eukaryotic chromosome.

1 32. The plant of claim 24, wherein the replication cassette comprises at
2 least 200 base pairs of DNA that is at least 70% identical to chromosomal DNA in the
3 plant cell.

1 33. The plant of claim 32, wherein the replication cassette comprises at
2 least 200 base pairs of DNA that is substantially identical to chromosomal DNA in the
3 plant cell.

1 34. The plant of claim 24, wherein the replication cassette comprises a
2 recombination sequence.

1 35. The plant of claim 34, wherein the recombination sequence is a *lox*
2 sequence.

1 36. The plant of claim 24, further comprising a polynucleotide
2 encoding a sequence-specific recombinase operably linked to a promoter.

1 37. The plant of claim 36, wherein the sequence-specific recombinase
2 is the Cre recombinase.

1 38. The plant of claim 24, wherein the eukaryotic replication promoter
2 is tissue-specific.

1 39. The plant of claim 24, wherein the eukaryotic replication promoter
2 is constitutive.

1 40. The plant of claim 24, wherein the eukaryotic replication promoter
2 is meiosis-specific.

1 41. The plant of claim 24, wherein the eukaryotic replication promoter
2 is inducible.

1 42. The plant of claim 24, wherein the polynucleotide(s) encoding T7
2 RNA polymerase, T7 gene 4 protein, T7 DNA polymerase and TrxA each encode a
3 nuclear localization signal.

1 43. A replication system comprising a polynucleotide encoding each of
2 the following polypeptides: T7 RNA polymerase, T7 gene 4 protein, T7 DNA polymerase

3 and TrxA, wherein the polynucleotide encoding each polypeptide is operably linked to a
4 eukaryotic replication promoter.

1 44. The replication system of claim 43, further comprising a
2 polynucleotide encoding a sequence-specific recombinase.

1 45. The replication system of claim 44, wherein the sequence-specific
2 recombinase is the Cre recombinase.

1 46. A polynucleotide, comprising
2 a bacteriophage T7 origin of replication;
3 a recombination sequence; and
4 an expression cassette comprising a eukaryotic replication promoter.

1 47. The polynucleotide of claim 46, wherein the recombination
2 sequence is a *lox* sequence.

1 48. The polynucleotide of claim 46, wherein the polynucleotide
2 comprises a T7 promoter.